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Effects of Chloroform in the Drinking Water of Rats and Mice: Ninety-Day Subacute Toxicity Study

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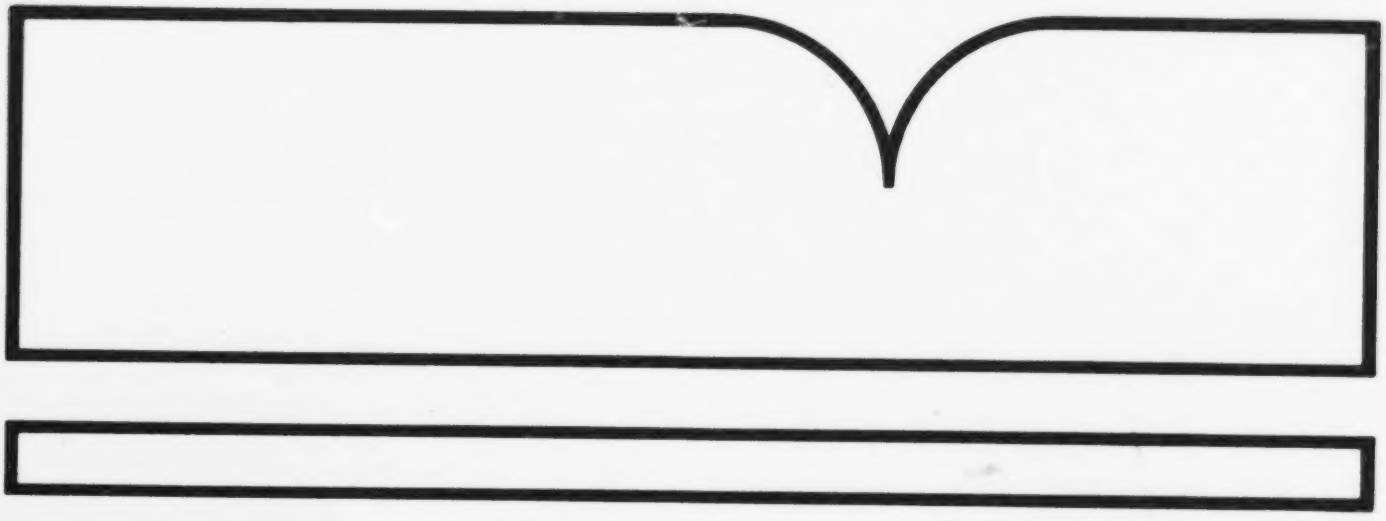

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Effects of Chloroform in the Drinking Water of Rats and Mice

Ninety-Day Subacute Toxicity Study

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16. ABSTRACT

This research was initiated to provide toxicologic and range finding data sufficient for setting dose levels for the chronic phase testing of chloroform in drinking water of male Osborne-Mendel rats and female B6C3F1 mice. A 90-day subchronic study was designed to investigate the effects of chloroform in drinking water at levels of 200, 400, 600, 900, and 1800 ppm in both the rats and mice, and 2700 ppm level in the mice. Body weights, water consumption, chloroform concentration in blood serum, kidney fat-to-kidney weight ratios (for rats), liver fat-to-liver weight ratios (in mice), and gross and microscopic pathology findings were examined.

Results showed that the body weights of both rats and mice receiving the high chloroform levels were affected by treatment, but the variances were low and the mice body weights returned to that of controls after one week. There was no effect on the percentage of kidney fat (rats) but a significant increase in liver fat (mice) was seen in the 2700 ppm group.

Data in this study indicated that all chloroform levels examined in the 90-day study are acceptable for use in the chronic study.

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EFFECTS OF CHLOROFORM IN THE DRINKING WATER OF RATS AND MICE
Ninety-Day Subacute Toxicity Study

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FOREWORD

The U.S. Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our national environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The primary mission of the Health Effects Research Laboratory in Cincinnati (HERL) is to provide a sound health effects data base in support of the regulatory activities of the EPA. To this end, HERL conducts a research program to identify, characterize, and quantitate harmful effects of pollutants that may result from exposure to chemical, physical, or biological agents found in the environment. In addition to the valuable health information generated by these activities, new research techniques and methods are being developed that contribute to a better understanding of human biochemical and physiological functions, and how these functions are altered by low-level insults.

In this report the results are presented from Phase I (the subchronic study) of the study on effects of chloroform in the drinking water of rats and mice. This phase was initiated to provide data for setting dose levels for the chronic-phase of testing chloroform in the drinking water of rats and mice. More reliable data is being sought on the chronic toxicity and carcinogenicity of chloroform to be used in risk assessment and to validate EPA standards for trihalomethanes in drinking water.

R. J. GARNER
Director
Health Effects Research Laboratory

ABSTRACT

This research program was initiated with the overall objective of providing toxicologic and range-finding data sufficient for setting dose levels for the chronic-phase testing of chloroform in drinking water of male rats and female mice.

A 90-day subacute study was designed to investigate the effects of five levels of chloroform (200, 400, 600, 900, 1800 ppm) in the drinking water of rats and six dose levels of chloroform (200, 400, 600, 900, 1800, and 2700 ppm) in the drinking water of mice. Body weight, water consumption, chloroform concentration in blood serum, kidney fat-to-kidney weight ratios, and gross and microscopic pathology findings were examined in rats. Body weight, water consumption, organ fat/organ weight ratios, and gross and microscopic pathologic findings were examined in mice.

Results showed that the body weights of rats receiving high chloroform levels were affected by treatment, but the variances were less than 20%. A dose-response was observed in water consumption, but intake at high chloroform levels did not drop below the generally accepted maintenance level of 25 ml per rat per day. No effect was observed on the percentage of kidney fat and on the chloroform concentration in serum. Gross and microsccpic pathology findings generally were slight or mild in severity, not dose-related, and either appeared adaptive (occurred in rats sacrificed after 30 or 60 days but not in those sacrificed after 90 days) or were sporadic and (by nature and/or incidence) not considered related to treatment.

Mice receiving 900, 1800, and 2700 ppm sustained body weight losses during the first week, but thereafter all body weights were comparable to those of controls. Considerable variability was evidenced in the water consumption, and as a result, a dose-dependency was not evident. The percentage of liver fat showed a statistically significant increase at the 2700 ppm level throughout the study. Gross pathological examinations revealed occasional hemorrhaging in the lungs of mice from all dose levels. Histologically, centrolobular fatty changes in mouse livers appeared related to treatment. Extramedullary hematopoesis in the liver and lymphoid atrophy of the spleen were also observed. Other lesions were sporadic and (by nature and/or incidence) not considered to relate to treatment.

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SECTION 1

INTRODUCTION

An aspect of environmental pollution of concern to the Environmental Protection Agency is the purity, acceptability, and wholesomeness of drinking water. In the determination of whether a problem exists, the first and most important step is to assess chemically the drinking water supplies in some major communities. This is being done, and, as the studies progress, the extent and magnitude of water pollution are revealed. Priorities must be established for further investigation, based on the concentrations of particular pollutants, their nature, and their potential for causing biologic harm.

One water pollutant, chloroform, is known to exist at concentrations of up to 0.3 ppm in almost all drinking waters that are disinfected with chlorine. A possible health hazard from this compound has been identified through a study completed for the National Cancer Institute by Hazelton Laboratories of America, Inc., of Vienna, Virginia. Chloroform was administered by corn oil to male and female Osborne-Mendel rats by gavage at levels of 90 and 180 mg/kg. These doses produced 8% and 24% incidences, respectively, of renal epithelial tumors in the male rats; no renal tumors were present in the females or in the controls. In a similar study with B6C3F1 mice, chloroform produced dose-dependent hepatocellular carcinomas. Little doubt remains that, under the conditions of these studies, chloroform can induce tumors in rats and mice. In other studies conducted at Huntingdon Research Center with rats, several strains of mice and dogs, somewhat lower doses produced renal tumors in only the male ICI-Swiss mice.

Even under the most extreme condition (0.1 mg/liter of $CHCl_2$ and 1 liter of drinking water drunk daily by a 10-kg child), the doses given rats in the Hazelton study were 9,000 and 18,000 times the normal human use level. For a 60-kg adult, the doses of 90 and 180 mg/kg are 22,500 and 45,000 times the human use level. Testing of animals with such high doses (maximum tolerated dose, MTD) is intended to facilitate identification of the target organs and to increase the possibility of lesions developing. However, the testing of animals at or near the use-level dose (with additional levels slightly increased to provide a safety factor) may provide more meaningful data and, ideally, a dose-response pattern.

Another point to emphasize is that in the Hazelton study, the laboratory animals received chloroform not in their drinking water, but by gavage in corn oil. This method of administration may have caused a strong daily chloroform pulse, and the corn oil may have enhanced absorption.

Other factors are prompting investigators to define and, it is hoped, solve the chloroform problem. Chlorination is viewed as the most effective means of ensuring a wholesome drinking water supply, although other methods are being investigated. Furthermore, a wide variety of other pollutants are being found in drinking water. Interactions of chloroform with one or more of these compounds may have biologic significance.

In the study reported here, we investigated the toxicity of chloroform administered in the drinking water of male Osborne-Mendel rats and female B6C3F1 mice for 90 days. The data derived from this study were used to select the dose levels for a program to investigate the carcinogenic potential of chloroform in the drinking water of male Osborne-Mendel rats and female B6C3F1 mice.

SECTION 2

CONCLUSIONS AND RECOMMENDATIONS

Chloroform can be put into solution at the levels being used in this program. Body weights of rats receiving higher dose levels were less than 20% below control weights. This difference should not affect the outcome of a chronic program adversely. The animals did continue to gain weight and appeared thrifty throughout. While a dose-response was evident in water consumption, intakes remained satisfactory for animal maintenance. The reduced water consumption did, however, slightly affect serum chemistry values. Organ fat (kidney) was unaffected by treatment. Also, chloroform does reach the blood stream and can be detected analytically at ppb levels. Gross and microscopic pathology findings generally were slight or mild, were not dose-related, and appeared adaptive or were sporadic and not considered related to treatment.

After an initial acclimation period, body weights and water consumption of mice were comparable to controls. There was a significant increase in liver fat for mice given chloroform at 2700 ppm. Gross pathological examination revealed no findings that could be attributed to treatment. Histopathologic examination showed extramedullary hematopoesis in the liver, lymphoid atrophy in the spleen and the centrolobular fatty changes in the liver. Other lesions were sporadic and (by nature and/or incidence) not considered treatment related.

All dose levels examined are suitable for inclusion in the chronic phase. We recommend that the 200, 400, 900, and 1800 ppm levels be used in the chronic phase of this program.

SECTION 3

EXPERIMENTAL PROCEDURES

COMPOUND SOURCE AND IDENTIFICATION

SRI purchased a sufficient quantity of pesticide-quality chloroform (Catalog # CX1052) from Matheson Coleman Bell (MCB). Lot No. 8G06 was used throughout this study. Analysis for purity showed conformity with MCB specifications. Approximately 30 ppb of diethyl carbonate (DEC) was detected during the purity analysis. Because the presence of DEC was of concern because of potential interference, the chloroform was distilled to remove DEC. This distillation was performed twice a week and was restricted to the quantities (100 ml) required for each dose-preparation period. Proceeding in this manner eliminated the need for routine monitoring of ethanol and phosgene concentrations.

PREPARATION OF CHLOROFORM WATER SOLUTIONS

Twice weekly, on Mondays and Thursdays, fresh aqueous solutions of chloroform were prepared and the old solutions were discarded. Each 10-liter bottle (with stirring bar) was numbered, and a record was kept of the empty and full weights, the calculated volume, and the amount of chloroform to be added to achieve the desired concentration. Each bottle was filled with distilled water to within several centimeters of the top and freshly distilled chloroform was added using volumetric pipettes and/or microliter syringes. The water level was brought to within 2.54 cm of the top, the bottle was stoppered, and the stopper was secured with duct tape. The bottle then was placed on a magnetic stirring unit (Vanlab Gyratherm UA/ #58922-054) for 16 to 24 hours of continuous mixing. No heat was used during the stirring process.

When solutions were thoroughly mixed, they were transferred to animal water bottles via a syringe-activated teflon siphon. For each chloroform level prepared, two samples were removed for analysis of chloroform content, the bottles were resealed and the solutions were saved for refilling any animal water bottles that spilled.

ANALYTICAL METHODS

Water Samples

Reagents:

Chloroform--from MCB, pesticide quality, distilled twice per week, lot analyzed by mass spectrometry.

Acetonitrile--for internal standard, from Burdick and Jackson, distilled in glass.

Water--filtered by reverse osmosis by a Milli-Pore Corporation Milli-Q purification unit, then filtered through a charcoal column (activated at 650°C for 12 hours), then continuously purged with filtered nitrogen gas (Molecular Sieve-13X) in a storage carboy.

Apparatus and Chromatographic Conditions

Gas Chromatograph (GD)--Varian model 2100 and Hewlett-Packard 5730.

Column--Chromosorb 101 Isothermal 140°C, 60/80 mesh, 6 ft (1.8 m) x 4 mm I.D.

Flame Ionization Detector (FID)--Attenuation Varian 2100, 1×10^{-11}, Attenuation HP 5730A, 1 Range 10.

Integration--via Spectra-Physics Minigrator, Hewlett-Packard 3380A.

Standards

An internal standard solution of 50% acetonitrile in water was prepared.

Twenty milliliters of purified water was sealed in a Teflon-capped vial with a crimp-on metal ring. A small magnetic stirring bar was in the vial. Chloroform was injected into the vial and dissolved by magnetic stirring (0.5 hour), then 5 µl of the internal standard was added. Standard concentrations of chloroform were 200, 400, 600, 900, 1800, 2700 ppm, and that of acetonitrile was about 100 ppm.

Samples

Samples were contained in sealed 20 ml vials. Five microliters of internal standard solution was added to give a final acetonitrile concentration of 100 ppm. Both samples and standards were shaken thoroughly to disperse the internal standard throughout the solution.

Results

The FID responsiveness of acetonitrile was about 5 times that of chloroform. The R_f value, defined as

$$R_f = \frac{\text{Area } CHCl_3}{\text{Area } CH_3CN} \cdot \frac{\text{ppm } CH_3CN}{\text{ppm } CHCl_3} ,$$

was about 0.175. For quantitation of samples, an area ratio was defined as:

$$\text{area ratio} = \frac{\text{Area } CHCl_3}{\text{Area } CH_3CN} \cdot \frac{\text{ppm } CH_3CN}{100} .$$

The division by 100 kept the numbers between 1 and 20.

Area ratio was plotted as a function of $CHCl_3$ (ppm) for the standards. The inverse slope of this plot (which was always linear) gave a factor--ppm $CHCl_3$/area ratio--that, when multiplied by the area ratio for the samples, would give ppm $CHCl_3$. Blanks always gave an area ratio of zero. The inverse of the slope factor, area ratio/ppm $CHCl_3$, times 100 gives an R_f value for all points on the best straight line through the standard points. The R_f value of standards prepared every week did not change by more than 5%.

Serum Samples

Reagents

Chloroform--same as that for water samples.

Bromochloromethane--from Aldrich Chemical Co.

Silicon Defoamer--Dow Corning, purchased from Arthur Thomas Co. Heated at 80%C under reduced pressure for 20 min to remove volatiles that would interfere with the GC analysis. The defoamer then was purged overnight at room temperature with filtered nitrogen.

Out-of-Date Human Donor Serum--from American Red Cross. Purged to remove volatile halogenated compounds and used for spiked standards.

Apparatus and Chromatographic Procedure

Purge Vessel--a) Standard EPA vessel with glass frit (5 ml) and Carle gas valve; b) Hewlett-Packard Model 7675A Purge and Trap Sampler.

Trap--Tenax GC 60/80 mesh, 15 cm x 2-5 mm I.D.

Purge Procedure--a) 95-100°C in oil or sand bath, 10 min with N_2 or He at 60 cc/min; b) room temperature, 10 minutes at 60 cc/min N_2.

Trap Desorption--2 min at 200°C.

GC Column--0.2% Carbowax 1500/Carbopack C 60/80, held at 20°C during desorption, then increased to 160°C at 8-10°C/min.

Electron Capture Detector (ECD)--a) ScH^3, Analog Technology Corporation Model 140A, linear pulse, 250°C; b) Ni^{63}, Hewlett-Packard, linear pulse, 250°C.

GC--a) Varian 2100, N_2 flow rate 37 cc/min; b) Hewlett-Packard 5730A, N_2 flow rate 37 cc/min.

Integrator--a) Spectra-Physics Minigrator; b) Hewlett-Packard 3380A.

Attenuation--a) Base current 230, attn 2^{14}; b) attn 1.

Standards

An internal standard solution of $ClCH_2BR$ in water (0.28 ppm) was prepared in a sealed vial.

A chloroform standard solution in water (0.10 ppm) was prepared in a separate vial.

Standards for purging were prepared by injecting 20 µl of the internal standard solution and 5, 10, 15, 20, and 25 µl of chioroform standard solution into 2 ml of purified water. Then 0.10 to 0.50 ml of human donor serum was added to keep the viscosity and surface tension the same as those in the sample solutions. Two to three drops of defoamer were added, the solution was purged onto the trap, and then desorption onto the analytical column was accomplished by heating. The column was temperature-programmed to elute the volatiles.

Samples

Serum samples were stored frozen. Just before analysis, they were thawed at room temperature. Then 0.10 to 0.50 ml of thawed sample was added to 2 ml of purified water containing 20 µl of the internal standard solution, 2 to 3 drops of defoamer were added, and the resulting solution was purged.

Results

Data were treated similarly to those for water samples. The area ratio was defined as:

$$\text{Area ratio} = \frac{\text{Area } CHCl_3}{\text{Area } ClCH_2Br} \cdot \text{nanograms } ClCH_2Br \text{ in solution.}$$

This area ratio was calculated for the standard solutions and plotted as a function of $CHCl_3$ (ng) in solution. Again the inverse of the slope gives ng $CHCl_3$/area ratio. When a blank containing 2 ml of purified water, 2 to

3 drops of defoamer, and 20 µl of internal standard solution was purged and chromatographed, the area ratio was not zero, which means that some chloroform was in the samples. Therefore, the area ratio of the blank was subtracted from the area ratios of the samples. This "corrected" area ratio then was multiplied by the slope factor to give nanograms of $CHCl_3$ present in the serum. Nanograms of chloroform present divided by ml of serum taken for purging gave ppb chloroform.

Standards were run every day or every other day and were run for heated and unheated purges (no differences in recovery or R_f were noted).

Chloroform in Air Samples

The same principles used above were applied to the analysis of chloroform in air samples in the animal rooms. Air samples were drawn through a Tenax trap by a calibrated metering pump and desorbed into a gas chromatograph as described above.

Organ Fat

Chloroform increases lipids in liver and kidney tissues, so monitoring this parameter in test animals is important. The procedure used to perform this assay was as follows. The organ tissue was tamped dry on the exterior and weighed on an analytical balance. The tissue then was homogenized with 4 ml of high-purity water (Milli-Q) using a Tektron, Inc., Polytron homogenizer. The homogenate was added to a separatory funnel containing 49 ml of chloroform:methanol, 2:1, the mixture shaken for 30 seconds, and 8 ml of 0.018 N H_2SO_4 added. The resulting mixture was shaken for 15 seconds, and the total contents were added to a 150 ml centrifuge bottle, which was spun at 2000 rpm for 20 minutes. The resulting suspension contained two layers separated by a thin white protein disk. The upper aqueous layer was drawn off by suction, and a 20 ml fraction of the bottom layer (chloroform) was evaporated to dryness in a tared 3 g test tube in a water bath at 57°C. Nitrogen gas was used to remove final traces of the solvent. The dry test tube was placed in a dessicator overnight, and the net weight, representing lipids, was determined.

Chloroform in Feed

The method used involved vacuum distillation of a feed and water slurry. The distillate was collected in a dry ice/acetone cold trap (-77°C) and a liquid nitrogen cold trap (-192°C). Chloroform solidifies at -63°C. The contents of the traps then were rinsed out with cold water, and the water was analyzed for chloroform by the purge-trap technique.

An aliquot of feed was ground up using a Waring blender. Ten grams were added to a 250 ml boiling flask containing 25 ml of high-purity organic-free water. The contents were heated (while being stirred) to 85°C for 1 hour. The vacuum was applied by means of a water aspirator.

At the end of the distillation, each trap was rinsed out with 25 ml of high-purity water.

The water rinse was analyzed by a purge trap using a Hewlett-Packard 7675A Purge and Trap Sampler with a Tenax Trap. The trap was desorbed at 150°C. For the analysis a Hewlett-Packard 7430A GC containing Carbowax 1500 (0.22)/Carbopack C column was used. The temperature was programmed to increase from 30°C to 160°C at 8°C/min, the detector was a Ni^{63} electron capture detector (ECD) and the purge gas and carrier gas was nitrogen.

Pesticides, PCBs, and Phthalates in Feed

Feed (Purina Laboratory Chow) was analyzed and cleaned up in two fractions. Each sample was analyzed by gas chromatography using ECD for the chlorinated pesticides and PCBs, and a nitrogen phosphorus specific detector for the organophosphorus pesticides. A packed column was used to separate components. The results of this analysis indicated that malathion was present. To confirm its presence, GS-mass spectrometry analysis was done. Only phthlates (dibutyl and dioctyl) were found. Other compounds searched for included p,p'-DDT, mirex, methoxychlor, endrin, dieldrin, aldrin, lindane, malathion, and aroclor 1254.

ANIMALS AND HOUSING

SRI purchased 250 male Osborne-Mendel rats from Camm Research Institute, Wayne, New Jersey, and received 300 female B6C3F1 mice from the Charles River Breeding Laboratory, at the request of NCI. The rats were born on 9/4/78 and received at SRI at 4 weeks of age on 10/3/78; the mice were born on 9/5/78 and received at SRI at 4 weeks of age on 10/4/78. After two weeks of quarantine, they were released for the tests at six weeks of age.

Rats were housed two per cage in 19" x 10-1/2" x 8" polycarbonate cages containing hardwood chip bedding and the mice were housed five per cage in 19" x 10-1/2" x 6" polycarbonate cages. Cages were changed twice a week for the rats and once a week for the mice by the biological technicians at the time the water bottles were changed. At the same time, each cage was rotated two positions to the right on the rack shelf and each rack was moved one position clockwise in the room. In this way, no cage was located in the same position on the rack during the 13-week study, and all racks were in each room position at least twice.

Control animals were housed in one room; chloroform-treated animals were housed in a similar but separate animal room. The temperature and humidity of each room was monitored by a hygrothermograph (Model #311, Weather Measure Corporation, Sacramento, California).

ANIMAL GROUPS AND DOSE LEVELS

The rats and mice were assigned to experimental groups by the following procedure. The animals were numbered consecutively. Odd-numbered rats had a picric acid mark on the back; even-numbered rats were unmarked. The mice were distinguished by different colored felt pen markers on the tail. Cage cards identified each pair of rats and group of mice. A table of random numbers was used to select the animal to be allocated to experimental groups. As each was selected, it was transferred to the appropriate labeled test

cage. The first rat in each cage received one notch clipped in the right ear; the second rat did not. Thus, the two rats assigned to each cage were distinguishable. The five mice in each cage were distinguished by the first mouse having one notch in the right ear; the second mouse having two notches in the right ear; the third mouse having one notch in the left ear; the fourth mouse having two notches in the left ear; and the fifth mouse having no notches in either ear.

The first two rats and five mice selected using the table of random numbers were assigned to the first cage of group 1, and the allocation proceeded until all cages in group 1 had been filled. Group 2 then was allocated in a like manner, and so forth until all groups were complete.

The test groups, levels of chloroform, and the number of animals assigned to each were:

Group No.	Dose Level (ppm)	No. of Animals
1	Ø (Control)	*80 (40 rats; 40 mice)
2	Ø (Matched Control)†	60 (30 rats; 30 mice)
3	200	60 (30 rats; 30 mice)
4	400	60 (30 rats; 30 mice)
5	600	60 (30 rats; 30 mice)
6	900	60 (30 rats; 30 mice)
7	1800	60 (30 rats; 30 mice)
8	2700	30 mice

* Includes 10 of each strain for day Ø sacrifice.

† See text under "Observations" for explanation.

OBSERVATIONS

Each animal was observed daily for abnormalities in appearance, activity, and general behavior. When an animal was moribund or lost weight steadily, it was observed closely throughout the day, and when it appeared unlikely to survive until the next day, it was sacrificed and necropsied.

Twice each week, bottles of freshly prepared chloroform/water solutions were placed on each cage. Bottle weights were recorded at the beginning and end of each semi-weekly measurement period. The difference in bottle weight was recorded as grams of test solution consumed, and the average grams consumed per day was then calculated. An exception to this procedure was made for the matched control groups. For each cage receiving 1800 ppm

(rats), or 2700 ppm (mice), a corresponding Ø ppm (matched control) cage was put on test 1 day later. Although the water bottles containing 1800 ppm or 2700 ppm chloroform were changed only twice a week, they were weighed daily to calculate each day's water consumption. The amount consumed by the animals in each cage then was allotted to the corresponding matched control cage for the following day. When deaths caused uneven numbers of mice in matched cages, the allotment was determined by calculating the amount of 2700 ppm water consumed per mouse, and multiplying this by five for the mice in Ø ppm matched cage. When an allotment value was indeterminable (e.g. due to spillage), the previous day's allotment was used.

At the same time as the semi-weekly bottle change, animals were transferred to clean cages containing new bedding.

Once each week, during the bottle-change procedure, samples were taken from five water bottles at each chloroform level, including Ø ppm. These samples were analyzed for chloroform concentration.

Once each week, on the alternate bottle-change day, animals were weighed and individual body weights were recorded. When an animal's weight seemed irregular, it was reweighed.

Ten rats and ten mice were sacrificed before the start of the study. The rats were used to determine baseline values of kidney fat-kidney weight ratios, serum chloroform, serum biochemistry, and gross and microscopic tissue pathology findings. The mice were used to determine baseline values of liver fat-liver weight ratios, serum LDH and SGOT activity, serum chloroform, and gross and microscopic pathologic findings.

Before all sacrifices, urine samples were obtained from 10 per group (10 total for the baseline group). The urine samples were evaluated for specific gravity, pH, protein, glucose, ketone, bilirubin, occult blood, and color. The specific gravity was measured on a refractometer, and the remaining measurements, except for color, were made using Ames Bililabstix.® All samples also were examined microscopically for casts, cells, crystals, and other inclusions.

Ten animals from each of the groups were sacrificed within several days after weeks 4, 8, and 13 of the test (days 30, 60, and 90). At each sacrifice period, every animal was first bled for analysis of chloroform in the blood, and the rats had serum analyses of the following:

SGOT	CO_2
SGPT	Cl^-
LDH	Albumin
BUN	Calcium
Triglycerides	Balance [$Na(Cl+CO_2)$]
Total cholesterol	Total bilirubin
Creatinine	Uric acid
Total protein	Globulin

Inorganic phosphorus	Total iron
Na^+	A/G ratio
K^+	Glucose
Alkaline phosphatase	

The mice were bled first for serum analyses of LDH and SGOT. Also, a peripheral blood smear was prepared, air-dried, and fixed in absolute methanol.

A complete necropsy was performed according to the Guidelines for Carcinogen Bioassay in Small Rodents (NCI-CG-TR-1, Technical Report Series #1, February 1976). The following tissues were examined and preserved in 10% neutral buffered formalin:

Gross lesions	Lungs and bronchi
Tissue masses	Heart
Suspect tumors	Thyroid glands
Regional lymph nodes	Parathyroid glands
Skin	Esophagus
Mandibular lymph node	Stomach
Mammary gland	Small intestine
Salivary gland	Spleen
Larynx	Kidneys
Trachea	Adrenals
Cecum	Bladder
Colon	Seminal vesicles
Rectum	Prostate
Mesenteric lymph node	Testes
Liver	Ovaries
Thigh muscle	Uterus
Sciatic nerve	Nasal cavity
Sternebrae, vertebrae, or femur (plus marrow)	Brain
	Pituitary gland
Costochondral junction, ribs	Eyes
Thymus gland	Spinal cord
Pancreas	Gall bladder

One kidney from each rat in the Ø, matched Ø, and 1800 ppm groups was reserved for determination of kidney fat/kidney weight ratios and a section of liver from each mouse in the Ø, matched Ø, and 2700 ppm groups was reserved for determination of liver fat/liver weight ratios as described previously.

Generally the following tissues were processed, stained, and examined microscopically:

Suspected tumors and gross lesions	Adrenal glands
	Spleen
Liver	Stomach
Kidney	Small intestine
Regional lymph nodes	Colon

Urinary bladder
Esophagus
Pancreas
Trachea
Seminal vesicles

Lung
Thymus
Testes

SECTION 4

RESULTS AND DISCUSSION

Analysis of the chloroform for identity and purity showed that we had received chloroform and that its purity met the manufacturer's specifications. During this analysis, we found 30 ppb of diethyl carbonate (DEC). Subsequently, each aliquot of chloroform used in the preparation of the drinking water was subjected to another simple distillation just before it was placed in solution.

Tables 1 and 2 present the results of analyses of samples taken from the first chloroform stock preparations made each week and of samples from bottles collected each Friday from selected animal cages in each treatment group. Occasionally, the analysis of bottle samples yielded values lower than we would have preferred (see 1/3 and 11/10). Generally, however, all values were well within acceptable limits, taking into account the number of times each preparation was handled and allowing for a reasonable analytical error.

Table 3 summarizes the results of analyses of blood serum for chloroform concentrations. Unfortunately, the 90-day samples were inadvertently contaminated with extraneous chloroform before analysis. A review of the procedures and of other findings from the 90-day data indicates that the values are approximately tenfold greater than expected. A serum analysis is scheduled to be conducted when the rats are sacrificed at 90 days on the chronic study. This sampling should verify the interpretation we have made of the present data. We demonstrated, however, that rats receiving several levels of chloroform (200 to 1800 ppm) in their drinking water show chloroform in their blood after day 30 of the test. Analyses of air samples collected in animal rooms showed levels of chloroform ranging from 0.4 to 56.3 ppb. Additional analyses were invalid because the blank decomposed.

Table 4 provides organ fat as a percentage of organ weight data from mouse livers collected at the 30-, 60-, and 90-day sacrifices. The liver fat was increased significantly in mice at the 2700-ppm level at all sampling periods. The significant findings in the matched control group at 90 days is not totally understood and cannot be fully explained.

Table 5 provides the amount of lipid as a percentage of organ weight from rat kidneys collected after the 30-, 60-, and 90-day sacrifices. No changes in lipids were observed in rat kidneys from the 1800-ppm dose level. The only significant increase in percent lipids occurred in the matched control group from the 90-day sacrifice.

Analysis of the block Purina Laboratory Chow for chloroform showed trace amounts of chloroform. Levels observed were in the ppb range and were not considered capable of influencing the outcome of this program. Further analysis of feed for pesticides and PCBs showed only dibutyl and dioctyl phthalates. These are not considered capable of interfering with the results of this study.

During the first week of treatment the animals receiving chloroform appeared generally depressed. The higher the dose level, the more apparent was the depression. During this initial three weeks, seven mice died (one at 600 ppm, two at 900 ppm, and four at 2700 ppm). For these animals, we observed body weight losses down to 11 grams, then death. From our daily observations, we attributed these losses to a refusal to drink the chloroform-treated water.

Table 6 presents the average body weights for female B6C3F1 mice during the 90-day study. Significant body-weight losses were observed during the first week in the mice receiving 900, 1800, and 2700 ppm and in the matched control group. After this initial acclimation period, all body weights were generally comparable to controls even though there were scattered significant differences occurring during weeks 2, 3, and 6.

Table 7 presents the average body weights for the rats during the 90-day study. Only the 1800-ppm group and the matched control group showed body weight gains consistently lower than those of the controls throughout the study.

Food consumption, observed weekly throughout the program, was considered to be normal.

Table 8 presents the average water consumption for female mice. Considerable variability and acclimation to the levels of chloroform were evident during the first eight sampling periods. After the eighth period, consumption became more consistent. Because of the variability in consumption, dose-dependency was not evident. Using average body weight and average water consumption data to calculate the average mg/kg/day chloroform intake showed that the mice had actual intake levels of from 148 to 175% of the intended levels. We had intended to provide 20, 40, 60, 90, 180, and 270 mg/kg/day of chloroform in the drinking water of mice. Our observations showed that the B6C3F1 female mouse is extremely active and has a relative low body weight. This, combined with a relatively consistent water consumption, accounts for the actual chloroform intake being higher than that predicted at the beginning of the program.

Table 9 presents the average water consumption for male rats. For these rats, the average water consumption for the chloroform-treated and matched control groups was consistently less than that of the control group. The decrement appears to be dose-dependent. The greatest reduction occurred in the 1800-ppm group and, as expected, in the corresponding matched control group. Using average body weight and average water consumption data to calculate the average chloroform intake in mg/kg/day showed an extremely close correlation to intended chloroform levels. It was intended

to provide chloroform doses at 20, 40, 60, 90, and 180 mg/kg/day in the drinking water of rats, and the actual cumulative intakes were 20, 38, 57, 81, and 160 mg/kg/day.

Table 10 summarizes the SGOT and LDH for female mice. These data vary so much that their usefulness in this program is questionable; they should be reviewed thoroughly before they are included in the chronic phase.

Summary data for the serum chemistry analyses for rats are presented in Table 11. Generally, glucose, creatinine, BUN, Na^+, Cl^-, calcium, balance, cholesterol, total bilirubin, SGOT, SGPT, LDH, alkaline phosphatase, total iron, total protein, albumin, globulin, and A/G ratio values in the treated groups were comparable with control values. Further, blood urea nitrogen (BUN), creatinine, uric acid, Cl^-, K^+, calcium, inorganic phosphorus, cholesterol, total protein, SGPT, albumin, and A/G ratio all showed significant increases on at least one dose level (generally the 1800-ppm level but there were some at the 400, 600, and 900-ppm levels) during the study. Values, lower than those in controls, were observed for CO_2, triglycerides, and lactic dehydrogenase (LDH). These reductions were generally confined to the 1800-ppm level. However, triglycerides were reduced at the 600-ppm level after 30 days and in the 900-ppm group after 60 days. The various changes that occurred in the matched control group were consistent with those expected from animals deprived of normal fluid intake (restricted water consumption).

Evaluation of urinalysis data of the rats showed a slight reduction in pH for chloroform-treated groups when compared with that in the control and/or matched control groups. Changes in protein observed in the urine were consistent with the changes in overall water consumption among the different experimental groups.

Microscopic examination of the sediment showed a rare to occasional red and/or white cell in all groups; an occasional hyaline and/or granular cast; and the sperm, triple phosphate crystals, and amorphous phosphates and urates expected in rat urine.

Gross pathologic examinations on each mouse revealed a very slight hemorrhaging of the lungs of several animals at all treatment levels.

Histologic examination of selected mouse tissues revealed the following.

Centrolobular fatty change in mouse livers occurred most often at the two highest dose levels (1800 ppm and 2700 ppm) but appeared to be mild and reversible. This lesion was evident at 400 ppm at 30 days but not at 60 or 90 days. The lesion is related to treatment but the occurrences at 400 ppm at 30 days may have been adaptive, reversible, and transitory.

Extramedullary hematopoesis in the liver was observed at virtually all dose levels at 30 days, and also is <u>not</u> related to treatment.

Lymphoid atrophy of the spleen was seen in 2 animals each at 60 and 90 days in the 2700 ppm mice. This was also noted in one animal each at 600

and 900 ppm at 90 days. Relationship to treatment appears likely, but this lesion is mild and unimpressive.

Other lesions were sporadic and (by nature and/or incidence) not considered related to treatment. Table 12 provides a summary of the histopathological findings in mice.

Gross pathological examination of each rat revealed the following findings, which were scattered among all treatment levels (including controls): fibrin-like material in urinary bladder; slight to moderate hemorrhaging of thymus, lungs, stomach and lymph nodes; hydronephrosis; and lung congestion. Histopathologic examination revealed an embryonal nephroma in the kidney of one control rat and lymphocytic lymphosarcoma in the thymus of another control rat. Tubular hyperplasias usually occurred in the kidneys of rats at the lower dose levels. These were mild, small, and circumscribed. Hepatosis, nephropathy, and tracheitis were increased over controls at all sacrifice intervals. Most lesions were slight or mild in severity, were not dose-related, appeared adaptive (occurred in 30- or 60-day sacrifices but not in 90-day sacrifices), or were sporadic and (by nature and/or incidence) not considered related to treatment. Table 13 provides a summary of the histopathological findings in rats.

In summary, there were both similarities and differences in the effects of chloroform on the two species. During the first week of administration, treated rats and mice exhibited signs of depression and decreased body weight. In mice, the weight decrements were transient, with recovery evident beginning in the second week. In rats, significant weight decrements persisted longer, lasting throughout the study at the highest dose level. Water consumption was decreased initially in treated animals of both species. In mice, decreased water consumption persisted for only about four weeks, while in rats it continued throughout the study. Food consumption appeared to be unaffected by chloroform administration in both species. The only serum constituents evaluated in both species, SGOT and LDH, were also unaffected. Fat content as a percentage of target organ weight was increased in the liver in treated mice but was normal in the kidney of treated rats. The only treatment-related pathologic finding was the occurrence of fatty changes in the liver in the mice.

TABLE 1. RESULTS OF ANALYTICAL CHEMISTRY ANALYSES - RATS

Chloroform Level (ppm)	1978 10/17	10/24	10/31	11/7	11/14	11/21	11/28	12/5	12/12	12/19	12/26	1979 1/2	1/9	1/16
	Date Sample Taken													
	PERCENTAGE OF EXPECTED VALUE FOR CHLOROFORM IN PREP SAMPLES (Average of two samples)													
200	83.3	75.3	83.0	81.8	88.3	109.3	87.0	82.5	103.3	108.3	87.3	123.0	104.8	98.5
400	83.8	78.1	88.6	69.4	89.4	90.5	81.6	101.9	90.6	109.3	104.5	115.9	98.3	94.8
600	92.0	80.8	84.8	72.8	94.8	92.7	81.6	86.8	88.8	97.5	103.9	112.0	91.3	96.6
900	101.8	82.0	95.8	84.6	92.4	89.9	82.9	81.9	92.2	95.8	105.6	109.8	91.9	96.7
1800	84.6	85.4	97.0	84.5	91.7	95.1	82.4	84.9	80.6	93.9	98.7	111.5	90.5	95.3
	PERCENTAGE OF EXPECTED VALUE FOR CHLOROFORM IN BOTTLE SAMPLES*													
	10/20†	10/27	11/3	11/10	11/17	11/24	12/1	12/8†	12/15	12/22	12/29	1/5	1/12	
200	96.8	86.5	61.5	81.1	77.3	68.9	88.7	87.9	98.8	89.6	99.6	93.3	99.6	
400	82.6	83.1	63.1	64.9	77.1	70.6	85.3	92.3	78.2	90.6	104.2	88.4	90.1	
600	87.4	81.5	67.8	68.9	80.7	72.3	81.5	89.3	84.8	98.0	102.6	86.5	85.8	
900	87.4	82.2	67.3	78.0	80.5	71.8	74.7	80.4	80.9	96.3	101.6	88.6	89.6	
1800	88.5	83.8	66.8	80.5	77.1	75.9	66.5	86.8	74.5	88.9	94.2	85.4	83.6	

*The first 4 weeks represent an average of 10 samples, the next 4 weeks represent an average of 9 samples, and the remaining 5 weeks represent an average of 7 samples for all levels.

†Some samples were lost when refrigerator malfunctioned; some bottles froze and broke.

TABLE 2. RESULTS OF ANALYTICAL CHEMISTRY ANALYSES - MICE

Chloroform Level (ppm)	1978 10/17	10/24	10/31	11/7	11/14	11/21	11/28	12/5	12/12	12/19	12/26	1979 1/2	1/9	1/16
	Date Sample Taken													
	PERCENTAGE OF EXPECTED VALUE FOR CHLOROFORM IN PREP SAMPLES (Average of two samples)													
200	83.3	75.3	83.0	81.8	88.3	109.3	87.0	82.5	103.3	108.3	87.3	123.0	104.8	98.5
400	83.8	78.1	88.6	69.4	89.4	90.5	81.6	101.9	90.6	109.3	104.5	115.9	98.3	94.8
600	92.0	80.8	84.8	72.8	94.8	92.7	81.6	86.8	88.8	97.5	103.9	112.0	91.3	96.6
900	101.8	82.0	95.8	84.6	92.4	89.9	82.9	81.9	92.2	95.8	105.6	109.8	91.9	96.7
1800	84.6	85.4	97.0	84.5	91.7	95.1	82.4	84.9	80.6	93.9	98.7	111.5	90.5	95.3
2700	97.2	91.7	82.2	79.6	93.2	97.5	87.2	93.1	92.8	92.6	92.2	107.7	89.5	85.9
	PERCENTAGE OF EXPECTED VALUE FOR CHLOROFORM IN BOTTLE SAMPLES*													
	11/20†	10/27	11/3	11/10	11/17	11/24	12/1	12.8†	12/15	12/22	12/29	1/5	1/12	
200	96.8	86.5	61.5	81.1	77.3	68.9	88.7	87.9	98.8	89.6	99.6	93.3	99.6	
400	82.6	83.1	63.1	64.9	77.1	70.6	85.3	92.3	78.2	90.6	104.2	88.4	90.1	
600	87.4	81.5	67.8	68.9	80.7	72.3	81.5	89.3	84.8	98.0	102.6	86.5	85.8	
900	87.4	82.2	67.3	78.0	80.5	71.8	74.7	80.4	80.9	96.3	101.6	88.6	89.6	
1800	88.5	83.8	66.8	80.5	77.1	75.9	66.5	86.8	74.5	88.9	94.2	85.4	83.6	
2700	92.7	81.7	72.5	76.7	75.9	79.1	76.0	83.4	70.6	72.3	94.4	82.0	86.1	

*The first 4 weeks represent an average of 10 samples, the next 4 weeks represent an average of 9 samples, and the remaining 5 weeks represent an average of 7 samples for all levels except 2700 ppm. The 2700-ppm level represents 5 samples during the first 4 weeks, 4 samples during the next 4 weeks, and 2 samples during the last 5 weeks.

†Some samples were lost when refrigerator malfunctions; some bottles froze and broke.

TABLE 3. AVERAGE CHLOROFORM LEVELS IN RAT SERUM (ppb)

Group	30-Day (SD)†	60-Day (SD)	90-Day (SD)†
Ø Day Baseline Control	692.2		
Ø ppm	0.60 (0.19)	1.45 (0.93)	165.7 (110.28)
Ø ppm Matched Control	0.89 (0.34)*	0.96 (0.35)	182.2 (77.59)
200 ppm	0.86 (0.31)*	1.07 (0.86)	162.7 (91.03)
400 ppm	1.26 (1.30)	0.92 (0.39)	187.5 (162.40)
600 ppm	1.12 (0.30)**	1.15 (0.78)	257.8 (151.14)
900 ppm	7.18 (9.50)*	1.34 (1.02)	237.6 (154.61)
1800 ppm	4.17 (1.66)**	5.89 (10.52)	294.6 (283.24)

†Samples found to have been contaminated with extraneous chloroform.
*Significant at $P \leq 0.05$.
**Significant at $P \leq 0.01$.

TABLE 4. LIVER FAT AS A PERCENTAGE OF LIVER WEIGHT - MICE

Treatment Group	30-Day Sacrifice	60-Day Sacrifice	90-Day Sacrifice
Control	3.52	2.18	4.08
Matched Control	3.19	2.36	6.80**
2700 ppm	8.22**	5.56**	6.52**

**Significant at $P < 0.01$.

TABLE 5. KIDNEY FAT AS A PERCENTAGE OF KIDNEY WEIGHT - RATS

Treatment Group	30-Day Sacrifice	60-Day Sacrifice	90-Day Sacrifice
Control	3.24	3.62	2.82
Matched Control	3.10	3.31	4.07**
1800 ppm	3.33	3.67	3.28

**Significant at $P < 0.01$.

TABLE 6. AVERAGE BODY WEIGHTS OF FEMALE MICE RECEIVING CHLOROFORM IN THEIR DRINKING WATER (Grams)

Week of Test	Chloroform Concentration (ppm) 0	200	400	600	900	1800	2700	0 (Matched)
Initial	20 (30)†	18 (30)	19 (30)	18 (30)	18* (30)	20 (30)	19 (30)	20 (30)
1	20	20	20	19	17*	17*	14* (29)	16*
2	21	20	19	18* (29)	19 (29)	19*	19* (26)	19*
3	21	21	20	20*	19* (28)	20	20	19
4	21	22	22	21	21	21	22	22
5	22 (20)	22 (20)	22 (20)	22 (19)	22 (18)	22 (20)	23 (16)	22 (20)
6	23	23	22	22	22	22	22	21*
7	23	23	22	22	22	23	23	22
8	23	23	23	22	22	22	23	22
9	24 (10)	23 (10)	23 (10)	22 (9)	22 (9)	23 (10)	23 (8)	22 (10)
10	24	23	23	23	23	23	24	23
11	25	24	24	24	23	24	24	23
12	24	24	25	24	24	24	24	25
13	24	24	25	25	24	25	25	25

†Number of mice included in average. Reduction in numbers due to mortalities and interim sacrifices.

*Significant at $P \leq 0.05$.

TABLE 7. AVERAGE BODY WEIGHTS OF MALE RATS RECEIVING CHLOROFORM IN THEIR DRINKING WATER (Grams)

Week of Test	Chloroform Concentration (ppm) 0	200	400	600	900	1800	0 (Matched)
Initial	191 (30)†	186 (30)	186 (30)	186 (30)	187 (30)	186 (30)	193 (30)
1	233	228	228	223	221*	204*	207*
2	274	266	265	261	261	239*	248*
3	306	302	299	296	294	273*	274*
4	330	325	320	319	317	288*	303*
5	346 (20)	344 (20)	345 (20)	339 (20)	347 (20)	305* (20)	317* (20)
6	365	358	358	354	362	316*	327*
7	370	371	375	369	379	326*	340*
8	381	380	380	373	385	333*	349*
9	397	394	394	387	396	342*	354*
10	414 (10)	420 (10)	397 (10)	376 (10)	405 (10)	345* (10)	362* (10)
11	425	432	399	391	422	357*	371*
12	433	444	414	399	429	364*	380*
13	428	435	407	399	430	361*	377*

†Number of rats included in average. Periodic reduction to scheduled interim sacrifices.

*Significant at $P \leq 0.05$.

TABLE 8. AVERAGE WATER CONSUMPTION OF FEMALE MICE RECEIVING CHLOROFORM IN THEIR DRINKING WATER (Grams per Mouse per Day)

Sampling Period†	Chloroform Concentration (ppm)							
	0	200	400	600	900	1800	2700	0 (Matched)
1	4.0	2.1	2.0	0.8	0.6	0.6	0.6	0.6
2	4.6	4.1	3.6	2.9	2.1	1.8	1.2	1.2
3	4.3	3.7	3.0	2.7	2.7	2.9	2.3	2.3
4	4.6	3.9	3.2	3.1	3.0	2.8	3.4	3.3
5	3.8	3.7	3.9	3.5	3.0	2.9	3.8	3.8
6	5.3	4.1	3.6	3.5	3.1	3.0	3.4	3.4
7	5.1	3.5	3.9	3.2	2.4	2.9	3.0	3.0
8	4.9	3.9	4.2	2.9	3.9	3.2	3.6	3.6
9	4.7	4.3	4.2	3.6	3.3	3.0	2.8	2.8
10	3.9	3.2	3.5	3.4	3.2	3.4	3.5	3.5
11	4.2	3.9	3.1	3.0	3.1	3.0	3.4	3.4
12	4.3	4.1	3.9	3.6	3.1	3.3	3.2	3.1
13	4.1	3.2	3.2	3.2	3.5	3.5	3.4	3.4
14	4.4	4.1	3.9	3.4	3.4	3.7	3.6	3.6
15	4.5	4.2	3.8	3.6	3.4	3.4	3.2	3.2
16	4.4	4.1	3.7	3.6	3.6	3.8	3.7	3.9
17	4.2	3.0	4.7	3.6	2.7	3.4	3.4	3.5
18	4.1	4.1	3.5	3.8	3.6	3.6	3.6	3.6
19	4.2	4.6	3.8	3.2	3.4	3.6	3.6	3.6
20	4.1	4.2	4.3	3.5	3.8	3.7	3.8	3.8
21	4.2	3.1	3.2	3.4	3.1	3.3	3.3	3.2
22	4.3	4.7	4.0	4.0	3.8	3.8	3.7	3.7
23	4.0	3.7	3.6	4.0	3.6	3.6	4.1	4.1
24	3.9	3.9	3.7	3.6	3.2	3.9	3.4	3.5
25	3.7	4.2	3.8	3.6	3.5	3.3	3.5	3.5
26	3.9	3.4	3.4	3.7	3.3	3.7	3.6	3.6

†Odd-numbered sampling periods are recordings of 3 days of water consumption and even-numbered sampling periods are recordings of 4 days of water consumption. These correspond with the number of days water bottles remained on the cages without changing. The chloroform solutions were prepared twice weekly.

TABLE 9. AVERAGE WATER CONSUMPTION OF MALE RATS RECEIVING CHLOROFORM IN THEIR DRINKING WATER
(Grams per Rat per Day)

Sampling Period†	Chloroform Concentration (ppm)						
	0	200	400	600	900	1800	0 (Matched)
1	37.3	29.5	26.5	24.7	24.3	16.9	16.4
2	40.6	34.5	33.3	33.3	32.3	25.3	25.4
3	40.0	33.1	31.1	29.1	30.0	25.5	25.4
4	42.3	34.1	31.7	30.9	30.2	27.1	26.6
5	45.3	34.0	31.9	29.5	28.6	26.4	25.9
6	39.7	33.0	31.9	30.1	29.3	27.1	27.2
7	38.6	32.4	31.6	30.3	30.1	25.5	25.4
8	40.1	35.8	35.0	31.7	32.5	27.4	27.4
9	39.7	35.5	32.6	32.2	32.1	27.0	26.9
10	38.9	32.0	31.0	31.5	31.7	26.2	26.3
11	38.5	30.5	30.2	30.1	30.2	25.7	26.0
12	40.4	32.4	30.3	30.9	30.3	26.3	26.3
13	40.1	30.9	28.8	29.5	28.8	25.5	25.4
14	42.2	32.0	30.7	30.9	29.5	27.2	27.1
15	42.1	33.8	31.6	31.0	29.2	26.3	26.4
16	42.3	33.4	30.7	31.2	30.5	28.1	26.8
17	39.9	32.4	29.3	31.7	29.0	25.4	25.2
18	40.7	33.3	30.0	30.5	29.3	25.4	25.4
19	44.1	35.7	31.7	31.4	29.1	26.5	26.5
20	41.2	35.0	32.0	28.8	29.0	27.5	27.5
21	44.2	36.1	31.5	31.5	30.6	27.2	27.1
22	41.6	35.1	29.6	32.9	31.1	27.2	27.4
23	42.6	34.7	32.7	32.2	31.0	27.6	27.5
24	42.3	33.9	33.1	29.8	29.0	27.4	27.6
25	40.5	35.2	31.7	31.8	31.9	27.4	27.6
26	40.6	32.8	28.9	28.8	27.3	24.4	24.0

†Odd-numbered sampling periods are recordings of 3 days of water consumption and even-numbered sampling periods are recordings of 4 days of water consumption. These correspond with the number of days water bottles remained on the cages without changing. The chloroform solutions were prepared twice weekly.

TABLE 10. AVERAGE SGOT AND LDH VALUES FOR FEMALE MICE RECEIVING CHLOROFORM IN THEIR DRINKING WATER

Treatment Group (ppm)	SGOT (U/L)	LDH (U/L)	Treatment Group (ppm)	SGOT (U/L)	LDH (U/L)
Baseline (Day zero)					
0	918	47			
30-Day interim sacrifice			60-Day interim sacrifice		
0	328	909	0	623	1010
200	225	738	200	400	996
400	233	767	400	414	965
600	196	601	600	257	1114
900	246	913	900	383	1018
1800	298	898	1800	136*D	548**D
2700	156*D	653	2700	619	1318
0 (Matched)	522	1525*	0 (Matched)	349	1413*
90-Day terminal sacrifice					
0	353	1222			
200	609	1520			
400	201	767*D			
600	392	1197			
900	234	1160			
1800	176*D	731**D			
2700	190	765**D			
0 (Matched)	620*	1506			

*Significant at $P < 0.05$.
**Significant at $P < 0.01$.
D, decreased below controls.

Table 11

SUMMARY OF CLINICAL CHEMISTRY DATA FOR RATS RECEIVING CHLOROFORM IN THEIR DRINKING WATER
(Ten Rats per Treatment Group)

Parameter Examined	Day Zero Baseline--SD†	30-Day Sacrifice: Chloroform Concentration (ppm) 0--SD	200--SD	400--SD	600--SD	900--SD	1800--SD	0 (Matched)--SD
Glucose (mg %)	162 (12.06)	193 (35.12)	231 (72.89)	252 (56.58)*	242 (91.55)	172 (30.75)	220 (60.77)	240 (101.80)
BUN (mg %)	20 (1.70)	20 (1.69)	22 (2.55)	23 (2.88)**	22 (3.82)	25 (9.94)	26 (1.60)**	28 (9.50)*
Creatinine (mg %)	0.6 (0.05)	0.6 (0.09)	0.7 (0.10)	0.7 (0.11)*	0.7 (0.09)*	0.6 (0.12)	0.5 (0.11)	0.7 (0.13)
Uric acid (mg %)	2.0 (0.26)	2.2 (1.09)	2.9 (1.80)	5.5 (1.90)**	3.5 (2.25)	2.1 (1.25)	3.9 (2.32)	3.3 (2.04)
Na^+ (meq/liter)	141 (1.20)	142 (1.29)	142 (0.99)	142 (1.99)	141 (1.34)	141 (1.78)	142 (2.00)	145 (1.51)**
K^+ (meq/liter)	5.3 (0.34)	5.4 (0.76)	6.1 (1.59)	7.2 (1.49)**	6.3 (1.89)	5.4 (0.86)	7.4 (2.16)*	6.2 (1.59)
CO_2 (meq/liter)	27 (1.78)	29 (1.83)	28 (1.85)	26 (1.69)**	28 (1.83)	28 (2.42)	24 (2.98)**	26 (1.60)**
Cl^- (meq/liter)	98 (1.35)	95 (2.58)	95 (2.37)	96 (1.85)	97 (2.51)	98 (2.05)**	99 (2.07)**	100 (3.10)**
Calcium (mg %)	10.6 (0.20)	10.3 (0.31)	10.5 (0.65)	11.1 (0.55)**	10.7 (0.89)	10.3 (0.30)	10.8 (0.69)*	10.4 (0.71)
Inorganic phosphorus (mg%)	9.4 (0.45)	8.4 (0.75)	8.4 (0.89)	9.1 (0.49)*	8.3 (1.25)	8.2 (1.20)	9.7 (1.64)*	7.9 (0.90)
Balance $Na-[Cl+CO_2]$	17 (1.70)	19 (2.41)	19 (3.06)	20 (2.88)	17 (3.67)	16 (4.68)	20 (2.72)	17 (2.91)
Cholesterol (mg %)	77 (3.56)	76 (29.27)	69 (5.46)	82 (44.89)	70 (4.42)	108 (127.03)	75 (5.85)	100 (112.30)
Triglycerides (mg %)	37 (14.07)	87 (58.22)	80 (23.43)	96 (98.15)	60 (10.00)	313 (832.05)	43 (8.69)*	123 (210.43)
Total Bilirubin (mg %)	0.2 (0.04)	0.1 (0.04)	0.1 (0.03)	0.2 (0.05)	0.2 (0.05)	0.2 (0.08)*	0.2 (0)**	0.1 (0.05)
SGOT (mμ/ml)	66 (22.57)	174 (89.72)	202 (73.84)	144 (36.96)	161 (51.49)	151 (66.76)	251 (125.33)	166 (64.19)
SGPT (mμ/ml)	125 (72.60)	63 (21.13)	83 (33.05)	63 (10.63)	60 (17.40)	83 (63.58)	112 (53.08)	62 (16.39)
LDH (mμ/ml)	1388 (331.82)	1593 (503.83)	1651 (360.78)	1270 (224.75)	1577 (302.73)	1275 (359.54)	1616 (482.38)	1484 (287.41)
Alkaline phosphatase (mμ/ml)	616 (77.85)	264 (50.38)	254 (42.44)	285 (62.10)	266 (40.82)	249 (75.24)	277 (52.18)	230 (50.38)
Total iron (meq %)	423 (57.60)	260 (49.64)	243 (30.20)	301 (52.33)	293 (66.83)	264 (90.98)	283 (48.64)	230 (52.78)
Total protein (g %)	4.7 (0.10)	5.3 (0.25)	5.3 (0.20)	5.6 (0.25)**	5.5 (0.18)*	5.3 (0.32)	5.7 (0.28)**	5.6 (0.20)**
Albumin (A) (g %)	2.5 (0.05)	2.6 (0.19)	2.7 (0.10)	2.7 (0.22)	2.7 (0.11)*	2.6 (0.35)	2.9 (0.14)**	2.7 (0.29)
Globulin (G) (g %)	2.18 (0.06)	2.69 (0.20)	2.61 (0.11)	2.87 (0.22)	2.75 (0.11)	2.74 (0.25)	2.75 (0.16)	2.91 (0.28)
A/G	1.14 (0.03)	0.96 (0.10)	1.02 (0.02)	0.95 (0.11)	0.99 (0.05)	0.95 (0.15)	1.06 (0.05)*	0.84 (0.15)

†SD = Standard Deviation, in parentheses.
*Significant at $P \leq 0.05$.
**Significant at $P \leq 0.01$.

Table 11 (continued)

Parameter Examined	60-Day Sacrifice: Chloroform Concentration (ppm) 0--SD†		200--SD		400--SD		600--SD		900--SD		1800--SD		0 (Matched)--SD	
Glucose (mg %)	190	(51.20)	192	(72.29)	165	(33.95)	177	(25.52)	203	(47.24)	219	(94.92)	162	(21.11)
BUN (mg %)	22	(1.70)	23	(1.89)	22	(2.63)	23	(1.03)	23	(1.27)	25	(2.58)*	26	(1.66)**
Creatinine (mg %)	0.7	(0.07)	0.7	(0.08)	0.6	(0.09)	0.6	(0.07)	0.7	(0.10)	0.7	(0.12)	0.7	(0.09)
Uric acid (mg %)	1.8	(1.29)	2.2	(1.71)	1.8	(1.34)	1.4	(0.45)	2.5	(1.53)	2.8	(2.52)	1.9	(0.75)
Na^+ (meq/liter)	142	(1.20)	141	(2.81)	141	(1.96)	143	(2.64)	141	(4.02)	141	(2.67)	145	(3.26)*
K^+ (meq/liter)	5.3	(1.05)	5.8	(1.36)	5.5	(0.99)	5.2	(0.51)	6.0	(1.38)	6.3	(2.39)	4.8	(0.55)
CO_2 (meq/liter)	29	(2.18)	28	(2.10)	29	(3.59)	28	(2.67)	27	(3.68)	26	(2.81)*	28	(1.81)
Cl^- (meq/liter)	97	(2.46)	97	(3.06)	97	(2.38)	99	(2.72)	98	(2.80)	98	(2.84)	97	(3.07)
Calcium (mg %)	10.0	(0.45)	9.8	(0.59)	9.7	(0.45)	9.9	(0.22)	10.0	(0.39)	10.2	(0.70)	9.4	(0.37)*
Inorganic phosphorus (mg %)	7.0	(0.37)	6.7	(0.94)	6.7	(0.67)	7.1	(0.47)	7.6	(0.54)**	7.6	(1.15)	6.2	(0.43)**
Balance $Na-[Cl+CO_2]$	16	(3.06)	16	(2.88)	16	(3.17)	16	(2.76)	17	(2.36)	17	(2.91)	21	(1.90)**
Cholesterol (mg %)	62	(6.98)	68	(7.87)	71	(6.56)**	75	(10.20)**	78	(11.26)**	79	(9.55)**	67	(8.01)
Triglycerides (mg %)	77	(25.60)	68	(15.83)	86	(20.67)	81	(30.29)	62	(24.73)	36	(15.45)**	53	(13.28)*
Total bilirubin (mg %)	0.1	(0.03)	0.1	(0)	0.1	(0.03)	0.1	(0.04)	0.1	(0.05)	0.2	(0.05)**	0.1	(0)
SGOT (mμ/ml)	138	(48.42)	129	(28.39)	117	(23.98)	122	(37.18)	124	(45.55)	104	(30.03)	142	(34.74)
SGPT (mμ/ml)	65	(17.89)	66	(12.83)	64	(15.17)	66	(16.58)	65	(13.20)	65	(19.87)	65	(11.29)
LDH (mμ/ml)	1370	(442.24)	1370	(368.27)	1190	(349.15)	1079	(328.35)	1014	(460.63)	676	(269.17)**	1467	(318.89)
Alkaline phosphatase (mμ/ml)	204	(36.81)	232	(45.56)	226	(41.50)	191	(38.92)	202	(55.39)	196	(30.99)	177	(22.68)
Total iron (meq %)	209	(27.35)	199	(22.00)	197	(15.52)	211	(22.76)	229	(27.71)	235	(38.90)	179	(19.97)*
Total protein (g %)	5.4	(0.24)	5.4	(0.22)	5.5	(0.11)	5.5	(0.24)	5.4	(0.31)	5.5	(0.21)	5.4	(0.27)
Albumin (A) (g %)	2.6	(0.15)	2.6	(0.11)	2.6	(0.11)	2.6	(0.14)	2.7	(0.21)	2.8	(0.11)*	2.7	(0.09)
Globulin (G) (g %)	2.78	(0.14)	2.82	(0.12)	2.87	(0.07)	2.84	(0.16)	2.71	(0.12)	2.76	(0.13)	2.70	(0.21)
A/G	0.94	(0.06)	0.91	(0.02)	0.92	(0.05)	0.93	(0.06)	0.98	(0.05)	1.01	(0.05)*	1.00	(0.06)

†SD = Standard Deviation, in parentheses.
*Significant at $P \leq 0.05$.
**Significant at $P \leq 0.01$.

Table 11 (concluded)

Parameter Examined	Terminal Sacrifice: Chloroform Concentration (ppm) 0--SD†		200--SD		400--SD		600--SD		900--SD		1800--SD		0 (Matched)--SD	
Glucose (mg %)	196	(43.11)	176	(40.10)	190	(64.15)	171	(36.55)	194	(41.86)	214	(83.79)	263	(118.20)
BUN (mg %)	22	(0.79)	22	(2.04)	22	(1.60)	23	(1.97)	22	(2.73)	25	(1.51)**	25	(4.59)
Creatinine (mg %)	0.6	(0.08)	0.6	(0.08)	0.6	(0.07)	0.6	(0.10)	0.6	(0.10)	0.7	(0.09)*	0.9	(0.15)**
Uric acid (mg %)	1.8	(1.00)	1.7	(0.36)	2.2	(2.06)	1.4	(0.77)	1.6	(1.52)	3.1	(1.79)	3.7	(2.31)*
Na^+ (meq/liter)	143	(0.95)	143	(1.37)	142	(1.43)	142	(0.99)	141	(1.43)**	141	(2.23)	145	(3.00)*
K^+ (meq/liter)	5.1	(0.75)	5.2	(0.91)	5.5	(1.40)	5.2	(0.63)	6.1	(2.02)	7.1	(2.64)*	6.9	(2.12)*
CO_2 (meq/liter)	27	(1.65)	28	(1.06)	27	(1.65)	27	(2.26)	27	(2.07)	26	(2.28)	24	(2.49)**
Cl^- (meq/liter)	97	(2.91)	97	(1.51)	98	(2.32)	99	(1.83)	98	(3.85)	98	(2.94)	104	(2.65)**
Calcium (mg %)	9.4	(0.33)	9.6	(0.30)	9.7	(0.51)	9.7	(0.25)*	9.9	(0.51)*	10.4	(0.79)**	9.9	(0.80)
Inorganic phosphorus (mg %)	6.4	(0.79)	6.1	(0.51)	6.3	(0.70)	6.4	(0.73)	6.8	(0.72)	7.7	(18.13)	5.8	(0.55)
Balance $Na^-[Cl+CO_2]$	18	(2.79)	17	(2.10)	16	(2.62)	17	(2.59)	16	(3.92)	17	(3.68)	18	(5.29)
Cholesterol (mg %)	62	(15.59)	73	(10.59)	75	(18.76)	73	(10.80)	76	(8.72)*	86	(12.69)**	87	(43.21)
Triglycerides (mg %)	78	(32.83)	93	(42.80)	91	(53.24)	80	(24.34)	93	(18.14)	38	(21.41)**	84	(45.94)
Total bilirubin (mg %)	0.1	(0.04)	0.1	(0.05)	0.1	(0.05)	0.1	(0.03)	0.2	(0.04)	0.2	(0.05)	0.1	(0.04)
SGOT (mμ/ml)	155	(88.50)	107	(17.48)	110	(34.53)	119	(38.91)	107	(18.37)	153	(51.18)	183	(81.24)
SGPT (mμ/ml)	62	(7.35)	59	(7.32)	72	(32.89)	64	(14.06)	61	(15.69)	84	(36.67)	74	(20.64)
LDH (mμ/ml)	2016	(1581.98)	1859	(699.94)	1547	(455.41)	1091	(299.91)	818	(404.95)	1016	(390.87)	1383	(592.22)
Alkaline phosphatase (mμ/ml)	195	(38.09)	192	(42.99)	202	(33.31)	186	(28.74)	177	(20.35)	160	(44.52)	165	(31.97)
Total iron (meq %)	195	(14.32)	194	(19.93)	215	(28.34)	204	(17.25)	226	(28.56)**	216	(17.17)**	175	(33.52)
Total protein (g %)	5.4	(0.20)	5.5	(0.16)	5.6	(0.20)	5.3	(0.24)	5.4	(0.21)	5.5	(0.24)	5.7	(0.22)**
Albumin (A) (g %)	2.7	(0.11)	2.7	(0.12)	2.7	(0.14)	2.6	(0.12)	2.6	(0.11)	2.8	(0.14)*	2.7	(0.23)
Globulin (G) (g%)	2.77	(0.18)	2.79	(0.19)	2.88	(0.12)	2.73	(0.16)	2.80	(0.16)	2.73	(0.14)	3.02	(0.29)*
A/G	0.96	(0.08)	0.97	(0.09)	0.93	(0.06)	0.94	(0.05)	0.95	(0.06)	0.93	(0.06)*	0.90	(0.13)

†SD = Standard Deviation, in parentheses.
*Significant at $P \leq 0.05$.
**Significant at $P \leq 0.01$.

Table 12

SUMMARY HISTOPATHOLOGICAL FINDINGS IN MICE

Microscopic Finding	Base-line	30-Day Sacrifice								60-Day Sacrifice								90-Day Sacrifice							
		Chloroform Concentration (ppm)								Chloroform Concentration (ppm)								Chloroform Concentration (ppm)							
	0	0	200	400	600	900	1800	2700	Matched 0	0	200	400	600	900	1800	2700	Matched 0	0	200	400	600	900	1800	2700	Matched 0
Lung																									
Hemorrhage	2									1			1								1	1			
Congestion																	1								
Liver																									
Extramedullary hematopoiesis	1	4	4	2	1			2	3																
Centrilobular fatty change				3		2	5	6								5							4	2	
Focal necrosis													1			1				2					
Focal autolysis														1											
Kidney																									
Tubular cyst																		2							
Focal congestion				1																					
Focal nephritis		1																							
Spleen																									
Lymphoid atrophy														1		2					1	1		2	
Adrenal																									
Extracapsular adrenal tissue	1																								
Stomach																									
Lymphoid infiltration				1																					

Table 13

SUMMARY HISTOPATHOLOGICAL FINDINGS IN RATS

Microscopic Findings	Baseline	30-Day Sacrifice							60-Day Sacrifice							90-Day Sacrifice						
		Chloroform Concentration (ppm)							Chloroform Concentration (ppm)							Chloroform Concentration (ppm)						
	0	0	200	400	600	900	1800	Matched 0	0	200	400	600	900	1800	Matched 0	0	200	400	600	900	1800	Matched 0
Lung																						
Alveolar collapse	1						2		1	3	1	1	2	2			1		1	1		
Alveolar dilatation							2							1					1	1		
Congestion									1	1												
Hemorrhage										1	1	1	2	1	1		1		1	2		
Exudative pneumonia																				1		
Liver																						
Pericholangitis	1			1	1		1		1		1		1	2	2	2		1	2	1	1	1
Necrosis				1		1		1														
Hepatosis:																						
Centrilobular				1	2	2	2			3	2	3	2	1	2	4	3		5		4	5
Mid-zonal			3		2	2	2		1	6	4	3	3	2		5	6	4	1	1		
Peripherolobular				1	1	1	1						1				1	4		2		
Thyroid																						
Thyroiditis	1										1											
Duodenum																						
Congestion	1																					
Focal ectopic pancreas	2																					
Focal hemorrhage			1																			
Autolysis														1								
Ileum																						
Autolysis		1	1	3			1	3	2	2	1	1	1	2	2	1	2	1			2	2
Mucosal hemorrhage																			1			
Jejunum																						
Autolysis		1	1	1			1						1									
Stomach																						
Glandular dilatation							2		1	1		1	1		2				2		2	
Parakeratosis			1				1			1								1				

Table 13 (continued)

Microscopic Findings	Baseline	30-Day Sacrifice							60-Day Sacrifice							90-Day Sacrifice						
		Chloroform Concentration (ppm)							Chloroform Concentration (ppm)							Chloroform Concentration (ppm)						
	0	0	200	400	600	900	1800	Matched 0	0	200	400	600	900	1800	Matched 0	0	200	400	600	900	1800	Matched 0
Thymus																						
Hemorrhage		1	2													1						
Lymphosarcoma																						
Kidney																						
Casts	3	3	2	2			1	1		2		1	1	4	4			2	1	1	2	
Chronic interstitial nephritis	3	2	2		1			1				1	1	5	4			1		1	1	
Mineralization	1	2	2	1	2	3	3	1	1	3	5	3	3	4	6	2	3	2	2	3	3	
Pelvic hemorrhage	1											2			1	1		2				3
Tubular cyst	1					1										2		1				1
Adenomatous					1												1					
Cellular casts				1																		
Cyst		1																				
Glomerulosclerosis		1	2					1												1		
Healed infarction							1															
Hematuria							2					1										
Hydronephrosis				1	1							3	4			1						
Nephropathy--																						
Slight focal		5	6	6	8	6	10	7	8	7	7	5	7	4	5	5	3	3	5	8	8	6
Moderate focal		1	2	1	1	2		1	2	1	3	4	2		1	5	6	5	4			2
Marked focal																						2
Moderate diffuse		1		1		1																
Marked diffuse						1		1														
Nephrosis								1						1				1	1			
Papillary cystadenoma			1																			
Proteinuria		2		2			1	1		2				2				1	1		1	
Pyelitis			2			2	1	2			1	3	2	3	2				1	3		
Tubular cystic hyperplasia				1																		
Tubular dilatation				2				1											1			
Tubular hyperplasia			1		1		1						1				1					1
Embryonal nephroma															1							
Glomerular hyalinization										1												
Unilateral hypoplasia																						1

Table 13 (continued)

Microscopic Findings	Baseline	30-Day Sacrifice							60-Day Sacrifice							90-Day Sacrifice						
		Chloroform Concentration (ppm)							Chloroform Concentration (ppm)							Chloroform Concentration (ppm)						
	0	0	200	400	600	900	1800	Matched 0	0	200	400	600	900	1800	Matched 0	0	200	400	600	900	1800	Matched 0
Urinary Bladder																						
Autolysis					1																	
Chronic cystitis																1	1					
Serosal mineralization																						1
Lymph node																						
Hemorrhage		1	1									1										
Lymphoid hyperplasia		1																				
Pancreas																						
Interstitial pancreatitis						1		1	2													
Periductal inflammation				1																		
Duct hyperplasia																					1	
Periductal fibrosis																					1	
Subacute pancreatitis																				1		
Spleen																						
Lymphoid hyperplasia		5	1	2	4	2	4	3				1	1					1	2			
Peritonitis			1											1								
Increase pigmentation												1				1		2	2	3	3	
Congestion													1									
Siderotic plaque																				1		1
Testes																						
Hyperplasia						1																
Interstitial cell hyperplasia						1																
Trachea																						
Glandular ectasia		1		1																		
Hemorrhage				1																		
Tracheitis		1	4	1	2	4		2	1	2	4		1	4			6	2	3		3	3
Cystic gland																					1	
Glandular dilatation																					1	

Table 13 (concluded)

Microscopic Findings	Baseline	30-Day Sacrifice							60-Day Sacrifice							90-Day Sacrifice						
		Chloroform Concentration (ppm)							Chloroform Concentration (ppm)							Chloroform Concentration (ppm)						
	0	0	200	400	600	900	1800	Matched 0	0	200	400	600	900	1800	Matched 0	0	200	400	600	900	1800	Matched
Adrenal																						
Cortical nodular hypertrophy															1						1	
Cortical vacuolation												2		2		1		1		1	1	
Extracapsular cortical nodules																1						
Colon																						
Contained blood																				1		
Submucosal hemorrhage																					1	
Seminal Vesicles																						
Chronic vesiculitis															1							